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MINIREVIEW

Microbial radiation-resistance mechanisms

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Organisms living in extreme environments have evolved a wide range of survival strategies by changing biochemical and physiological features depending on their biological niches. Interestingly, organisms exhibiting high radiation resistance have been discovered in the three domains of life (Bacteria, Archaea, and Eukarya), even though a naturally radiation-intensive environment has not been found. To counteract the deleterious effects caused by radiation exposure, radiation-resistant organisms employ a series of defensive systems, such as changes in intracellular cation concentration, excellent DNA repair systems, and efficient enzymatic and non-enzymatic antioxidant systems. Here, we overview past and recent findings about radiation-resistance mechanisms in the three domains of life for potential usage of such radiation-resistant microbes in the biotechnology industry.

Keywords: radiation, DNA damage, reactive oxygen species, antioxidant mechanism, microorganism

Introduction

The ability to sense, respond, and adapt to the surrounding environment is critical for all living organisms in their biological niches. For example, microorganisms exposed to unfavorable conditions such as high salt stress and temperature harbor unique cellular remodeling systems and optimize their metabolic profiles to adapt to external cues (Stan-Lotter and Fendrihan, 2012). Radiation, which is the emission of energy in the form of electromagnetic waves from a natural source or caused by human activities, affects cellular biomolecules including nucleic acids, proteins, and lipids directly or indirectly (Halliwell and Gutteridge, 1999). For example, ionizing particles such as α-particles or neutrons directly interact with DNA, which damages the sugar backbone and the purine/pyrimidine base and thereby disrupts the DNA structure (Close *et al.*, 2013). This accounts for approximately 20% of the cytotoxic effects conferred by ionizing radiation on the cells (Halliwell and Gutteridge, 1999). In the indirect action, ionizing radiation produces reactive oxygen species (ROS) such as hydroxyl radical (OH•), superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2) through radiolysis of water. Subsequently, ROS interact with 2'-deoxyribose or nucleobases, and other cellular molecules, resulting in detrimental effects on cell survival (Halliwell and Gutteridge, 1999; Close *et al.*, 2013).

One of the radiation-induced fatal effects on living organisms is a chromosomal DNA lesion brought about by damaging nucleotide bases and introducing DNA single-strand breaks (SSBs) or double strand breaks (DSBs) (Azzam *et al.*, 2012). Among the DNA lesions, DSBs result in chromosomal aberrations, which cause genome instability and death in cells, if not appropriately repaired (Rich *et al.*, 2000; Hoeijmakers, 2001). ROS produced by ionizing radiation damage protein, lipids, nucleic acids, and carbohydrates. ROS are able to cleave the protein backbone and induce protein oxidation (Madian and Regnier, 2010). Carbonylation, which is the post-translational addition of carbonyl moieties to amino acid side chains leading to a loss of protein function by altering protein folding, increases after ionizing radiation exposure (Sukharev *et al.*, 1997; Maisonneuve *et al.*, 2009). Carbonylated proteins could be toxic if not properly degraded by the proteasomal system or folded by chaperone systems (Dalle-Donne *et al.*, 2006). To counteract these lethal effects, living organisms activate DNA repair machineries, facilitate enzymatic and non-enzymatic antioxidative systems, and induce protein folding and degradation systems.

Levels of ionizing radiation in the natural environment are not intensive. Nevertheless, a group of archaeal, prokaryotic, and eukaryotic organisms has been found to exhibit higher radiation resistance (1 to 10 kGy) than *Escherichia coli* (D_{10}: 0.2–0.7 kGy) and humans (D_{50}: 3 Gy) (Karam and Leslie, 1999; Confalonieri and Sommer, 2011). Organisms harboring the ability of radiation resistance have unique regulatory systems including production of secondary metabolites, antioxidative defense systems, and DNA repair machineries to counteract stress caused by radiation exposure (Gabani and Singh, 2013). Here, we review radiation-resistant mechanisms of prokaryotic, archaeal, and eukaryotic organisms to comprehensively understand their unique features compared to those in other radiation-sensitive organisms.

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Radiation-resistance mechanisms in bacteria

Ionizing radiation-resistant bacteria (IRRB) were defined as non-spore-forming bacteria for which more than 1 kGy of acute ionizing radiation is required to achieve 90% reduction (D_{10}) (Sghaier *et al.*, 2008). Diverse IRRB have been isolated from a wide range of environments (Rainey *et al.*, 2005). Among these, *Deinococcus*, *Kineococcus*, and *Rubrobacter* species were mainly isolated and show higher levels of resistance to radiation than other IRRB strains (Anderson, 1956; Ferreira *et al.*, 1999; Phillips *et al.*, 2002).

Deinococcus radiodurans, which was formerly named *Micrococcus radiodurans*, is a well-known IRRB that can survive in a higher dose of γ-radiation (D_{10}: 12 kGy) compared to *E. coli* (D_{10}: 0.2–0.7 kGy) (Daly, 2012). In addition to γ-radiation resistance, *D. radiodurans* exhibits high levels of resistance to UV-C irradiation (100 to 295 nm), desiccation, and oxidative stress (Slade and Radman, 2011). *D. radiodurans* was isolated from canned meat exposed to ionizing radiation for sterilization (Anderson, 1956). The fact that *D. radiodurans* shows extreme resistance to radiation renders this strain an attractive model system for investigation of the radiation-resistance mechanism. The remarkable radiation resistance of *D. radiodurans* is attributed to a myriad of cellular defense mechanisms, including efficient DNA repair systems and intrinsically high antioxidant activities (Cox and Battista, 2005; Daly, 2009). Early studies on *Deinococcus* hypothesized that *Deinococcus* has specialized DNA repair systems to counteract extreme radiation-mediated cellular stress. To prove it, a series of studies was conducted to identify a unique DNA repair system underlying radiation resistance of *Deinococcus* species by comparing their genomes to those of radiation-sensitive species (Lin *et al.*, 1999; Makarova *et al.*, 2001, 2007). Unexpectedly, however, DNA repair systems of *D. radiodurans* do not appear to be remarkably distinguishable from those of radiation-sensitive species. Indeed, the DNA repair systems in *D. radiodurans* are less complex than those of radiation-sensitive *E. coli* and *Bacillus subtilis* (Makarova *et al.*, 2001). For example, genes encoding DNA repair proteins, such as photolyases, translesion polymerases, and a DNA dioxygenase are not found in the *D. radiodurans* genome (White *et al.*, 1999). In contrast, *D. radiodurans* has a large number of DNA glycosylases involved in base excision repair (BER) (Norais *et al.*, 2009).

Notably, distinct features in the DNA repair system of *D. radiodurans* have been found through molecular functional analysis approach. Ensuing studies with transcriptome analyses revealed that *Deinococcus*-specific genes [*ddrA*, *ddrB*, and *irrE* (*pprI*)] exhibit increased expression in response to γ-radiation and mediate γ-radiation resistance (Earl *et al.*, 2002; Hua *et al.*, 2003; Liu *et al.*, 2003; Tanaka *et al.*, 2004). PprI, whose deletion increases γ-radiation sensitivity, regulates expression of *recA* (recombinase A) and *pprA* (a pleiotropic protein involved in DNA ligation) (Narumi *et al.*, 2004; Repar *et al.*, 2010). In line with these results, strains lacking *pprI* show delayed genome recovery compared to its wild-type strain post-radiation exposure (Lu *et al.*, 2009), indicating that the PprI-mediated DNA repair system is essential for radiation resistance of *D. radiodurans*. In addition, *D. radiodurans* has a diversity of DNA repair systems including extended synthesis-dependent strand annealing (ESDSA) process (Zahradka *et al.*, 2006). Newly synthesized, long, and single-stranded overhangs generated in the ESDSA process provide chances to reconstruct a functional genome from a number of chromosomal fragments caused by radiation exposure. Recent studies revealed that the RecFOR pathway plays critical roles in DNA DSB repairs through ESDSA process. Supporting this, strains devoid of RecF, RecO, or RecR exhibit susceptibility to γ-radiation (Bentchikou *et al.*, 2010).

γ-Radiation exposure increases protein carbonylation, which hampers catalytic activity of proteins and results in cell death, as H_2O_2 treatment does. This phenomenon suggests that antioxidant defense mechanisms of *Deinococcus* would also be critical for radiation resistance. Supporting this hypothesis, it has been found that *D. radiodurans* harbors intensive enzymatic and non-enzymatic antioxidant defense systems compared to radiation-sensitive bacteria (Daly, 2009). The red pigment, carotenoid, is an efficient scavenger of ROS such as single oxygen ($^1O_2$) and peroxyl radicals (ROO•) (Tatsuzawa *et al.*, 2000; Stahl and Sies, 2003). Deinoxanthin, which is a major product of the carotenoid synthesis pathway in *D. radiodurans*, has higher scavenging ability and protects DNA from oxidative stress to a greater extent than lycopene and β-carotene (Tian *et al.*, 2007). In agreement with these results, strains lacking genes involved in carotenoid synthesis exhibit increased susceptibility to γ-radiation (Tian *et al.*, 2007; Xu *et al.*, 2007; Zhang *et al.*, 2007). In addition, pyrroloquinoline-quinone (PQQ) plays critical roles in radiation resistance of this organism. PQQ is a redox cofactor for glucose dehydrogenases in bacteria (Duine, 1990). Recently, the roles of PQQ as an antioxidant have been demonstrated in bacterial systems (Khairnar *et al.*, 2003; Misra *et al.*, 2004). *D. radiodurans* strains lacking PQQ synthesis exhibit susceptibility to γ-radiation and DNA damage insults such as mitomycin C-induced damage (Rajpurohit *et al.*, 2008). *E. coli* expressing the *D. radiodurans* PQQ synthase gene exhibits increased resistance to oxidative stress (Misra *et al.*, 2004). Therefore, non-enzymatic antioxidants such as deinoxanthin and PQQ contribute to γ-radiation resistance of *D. radiodurans*.

In addition to non-enzymatic antioxidants, enzymatic activity of proteins involved in oxidative stress plays critical roles in radiation resistance of *D. radiodurans*. To relieve deleterious effects caused by ROS, cells exploit a series of enzymes such as superoxide dismutase and catalase to convert ROS into harmless molecules. Although the number of superoxide dismutases (1 SodA and 3 SodC in *D. radiodurans*; SodA, SodB, and SodC in *E. coli*) and catalases (KatE1, KatE2, and DRA0146 in *D. radiodurans*; KatE and KatG in *E. coli*) in *D. radiodurans* is almost equivalent to that in *E. coli*, the antioxidant enzymes in *D. radiodurans* show enhanced levels of activity in comparison to those in *E. coli*. Hua *et al.* (2003) showed that ROS scavenging activities of superoxide dismutase and catalase in *D. radiodurans* are much higher than those in *E. coli* (Tian *et al.*, 2004). In agreement with these results, deletion of catalase (KatE1) or superoxide dismutase (SodA) renders *D. radiodurans* sensitive to ionizing radiation (Markillie *et al.*, 1999). Therefore, the highly efficient enzyme-mediated antioxidant defense system

contributes to radiation resistance of *D. radiodurans*.

In *D. radiodurans*, the Mn^{2+} complex-mediated antioxidant defense system has been most extensively studied. Many researchers originally hypothesized that lesion-yield (number of DNA lesions per cell immediately after radiation exposure) for DSBs in *D. radiodurans* is much lower than that of radiation-sensitive bacteria. Surprisingly, however, the lesion-yield for DSBs (275 DSBs at D_{37}) of *D. radiodurans* was found to be much higher than that of radiation-sensitive species, *E. coli* K12 (8–9 DSBs at D_{37}) (Burrell *et al.*, 1971; Krasin and Hutchinson, 1977). In contrast, radiation-induced protein oxidation is much lower in *D. radiodurans* than in radiation-sensitive bacteria (Daly, 2009). These results imply that inactivation of repair proteins by radiation-induced oxidative stress is critical for survival of radiation-sensitive bacteria rather than DSB itself. These findings prompted many researchers to identify critical factors to affect protein oxidation in *D. radiodurans*. Interestingly, protein-free extracts from *D. radiodurans* increase the resistance of *E. coli* to X-rays, suggesting that small molecules in *D. radiodurans* extract could be radiation-resistant factors (Bruce, 1964). Daly *et al*. (2004) reported that *D. radiodurans* harbors higher levels of intracellular manganese, but lower levels of iron than radiation-sensitive bacteria : Mn/Fe ratios in *D. radiodurans* and *E. coli* are 0.24 and 0.0072, respectively. This intracellular Mn^{2+} accumulation is also observed in other IRRB, including *Enterococcus*, *Lactobacillus*, and cyanobacteria (Daly *et al.*, 2004). The Mn^{2+} accumulation prevents protein oxidation, not DNA damage, caused by radiation exposure. Ensuing studies further revealed accumulation of Mn^{2+}, inorganic phosphate, nucleosides, bases, and small peptides in the *D. radiodurans* ultrafiltrate (Daly *et al.*, 2010). Manganese and orthophosphate *per se* do not show protective activity for proteins exposed to γ-radiation, but the Mn^{2+}/orthophosphate complex exhibits protective effects by catalytically removing superoxide (Barnese *et al.*, 2008; Daly *et al.*, 2010). Furthermore, the mixture of orthophosphate and nucleosides prevents protein oxidation. The Mn^{2+}/peptide complex exhibits ROS scavenging activity (Berlett *et al.*, 1990; Daly *et al.*, 2010). Taken together, high intracellular concentration of manganese has an influence on the radiation resistance of *D. radiodurans* by formation of complexes with orthophosphate or peptide.

Kineococcus radiotolerans, which was isolated in a radioactive area at the Savannah River Site in Aiken, South Carolina, USA (Phillips *et al.*, 2002), exhibits high radiation resistance (D_{10} = 2 kGy), and is also highly resistant to oxidative stress and desiccation. Although the radiation-resistance mechanism of this strain has not been extensively investigated in this organism, recent studies have provided some insight into physiological factors and molecular mechanisms to detoxify damage caused by radiation exposure. Notably, *K. radiotolerans* uniquely employs divalent cations to enhance radiation resistance. Although *K. radiotolerans* shows similar Mn/Fe ratio compared to other IRRB, it has lower intracellular levels of Fe^{2+} (0.86 nmol/mg protein; > 1.4 nmol/mg protein in other IRRB) and Mn^{2+} (0.075 nmol/mg protein; > 0.3 nmol/mg protein in other IRRB) (Bagwell *et al.*, 2008b). Furthermore, *K. radiotolerans* supplemented by copper shows more enhanced survival rate than cells treated with other cations such as Fe^{2+}, Mn^{2+}, Mo^{2+}, and Zn^{2+} during chronic irradiation. Unexpectedly, manganese treatment to *K. radiotolerans* appears to confer lethal effects on its growth during chronic irradiation (Bagwell *et al.*, 2008b), which is in stark contrast to the case in *D. radiodurans*.

Several lines of evidence provided by other studies hypothesize that efficient DNA repair machineries are more important for radiation resistance of *K. radiotolerans* than ROS scavenging systems. First, genome analysis of *K. radiotolerans* revealed that genes involved in replication, repair, and recombination are enriched like other IRRB including *D. geothermalis*, *D. radiodurans*, and *Rubrobacter xylanophilus* (Sghaier *et al.*, 2008). Second, Li *et al.* demonstrated that although *K. radiotolerans* harbors a wide range of ROS scavenging related genes, only the *katA* gene (Krad_0815) increases in response to radiation exposure (Bagwell *et al.*, 2008a; Li *et al.*, 2015). Furthermore, according to gene cluster analysis based on the COG (Clusters of Orthologous Groups), a majority of genes up-regulated by radiation exposure belong to the group of DNA replication, recombination, and repair (Li *et al.*, 2015). Expression of *recA* (bacterial recombinase A), *ruvA*, and *ruvB* (Holliday junction DNA helicases) particularly increases post-radiation exposure. In line with the transcriptome analysis of *K. radiotolerans*, a large portion of small noncoding RNAs (sRNAs) including transcription regulation and DNA repair machinery are down-regulated when cells are exposed to radiation (Chen *et al.*, 2016). To further support the hypothesis, however, the radiation-regulated genes should be functionally characterized in future studies.

The thermophilic species - *Rubrobacter radiotolerans* and *R. xylanophilus*, which belong to the class Actinobacteria, exhibit high levels of radiation resistance (D_{10} of *R. radiotolerans*: 11 kGy; D_{10} of *R. xylanophilus*: 5.5 kGy) (Ferreira *et al.*, 1999). *R. radiotolerans*, formerly described as *Arthrobacter radiotolerans* (Suzuki *et al.*, 1988), was isolated from a radioactive hot spring in Japan (Yoshinaka *et al.*, 1973), whereas *R. xylanophilus* was isolated from a thermally polluted effluent in the United Kingdom (Carreto *et al.*, 1996). The radiation-resistant mechanisms of *R. radiotolerans* are distinguishable from those of other IRRB. The rate of DNA strand break by ionizing radiation is relatively lower in *R. radiotolerans* than in other IRRB: 2.03 DNA DSB per genome at 1 kGy of γ-radiation in *R. radiotolerans* (Terato *et al.*, 1999) and 7.5 DNA DSB per genome at 1 kGy in *D. radiodurans* (Kitayama and Matsuyama, 1971). Furthermore, intrinsic factors such as radio-protecting pigment contribute to the radiation resistance of *R. radiotolerans*. The carotenoid class of the reddish pigment, bacterioruberin, observed in *R. radiotolerans* confers radiation resistance (Saito *et al.*, 1994). In line with this result, *Halobacterium salinarium* strains lacking bacterioruberin exhibit increased sensitivity to γ-radiation, UV irradiation, and H_2O_2 (Shahmohammadi *et al.*, 1998). In addition, higher concentration of intracellular compatible solutes such as trehalose and mannosylglycerate is also regarded as a possible factor to make cells radiation-resistant.

Radiation-resistance mechanisms in archaea

Archaea exist in a wide range of extreme habitats including highly saline, acidic, or alkaline water. Among the extremophiles, *Thermococcus gammatolerans* was isolated after 30 kGy γ-radiation exposure from samples collected from a deep-sea hydrothermal vent chimney and was able to withstand the 3 kGy of radiation without loss of viability (Jolivet *et al.*, 2003a). The radioresistance of this strain does not depend on growth phases under optimal growth condition, but on the nutrition availability (Tapias *et al.*, 2009). A recent study revealed that unlike other archaea, *T. gammatolerans* does not have unique DNA repair genes (Zivanovic *et al.*, 2009). Instead, proteome analysis shows that proteins involved in the DNA repair machinery are constitutively induced. Furthermore, comparative genome analysis found that the *T. gammatolerans* genome harbors a number of novel genes involved in detoxification of radiation-mediated deleterious effects (Zivanovic *et al.*, 2009). The hyperthermophiles, *Pyrococcus furiosus* (D_{10}: 3 kGy) and *Pyrococcus abyssi* (D_{10}: 3 kGy), also show high levels of radiation resistance like *T. gammatolerans* (DiRuggiero *et al.*, 1997; Gerard *et al.*, 2001). Given that DNA damages induced by high temperature are similar to those induced by ionizing radiation, the intrinsic property of hyperthermophiles (the optimal growth temperature is nearly 100°C) might be responsible for γ-radiation resistance. Transcriptome analysis revealed that the radiation-resistance mechanisms of *Pyrococcus* species are similar to those of *T. gammatolerans* (Williams *et al.*, 2007). First, most genes related to DNA repair machinery are constitutively expressed by γ-radiation in *Pyrococcus* species. Supporting this, fragmented chromosomal DNA is fully restored within 2 to 4 h post-radiation exposure (2.5 kGy) in *P. abyssi* and *P. furiosus* (DiRuggiero *et al.*, 1997; Jolivet *et al.*, 2003b). Second, genes involved in ROS detoxification and redox homeostasis are constitutively expressed. For example, induction of proteins with ferritin-like di-iron motif post-radiation exposure could inhibit production of ROS by Fenton reaction.

The radiation-resistance mechanism of *H. salinarum*, which is a halophilic archaeon, has been also well addressed. It contains high concentration of inorganic cations (K^+) and possesses compatible solutes such as trimethylammonium compounds, which are well known osmotic regulators in diverse species, to survive in the extremely salty environment (Kokoeva *et al.*, 2002; Engel and Catchpole, 2005). In addition to osmotolerance, *H. salinarum* exhibits resistance to high doses of UV radiation, desiccation, and vacuum (Baliga *et al.*, 2004; Kottemann *et al.*, 2005). Particularly, it shows remarkable resistance to γ-radiation (D_{10} value: 5 kGy) (Kottemann *et al.*, 2005). Given that intracellular salts such as KCl mitigate toxic effects generated by oxidative free radicals (Marguet and Forterre, 1998; Shahmohammadi *et al.*, 1998), the high concentration of intracellular KCl renders this strain resistant to γ-radiation. Recent studies have revealed that additional non-enzymatic antioxidant systems also influence radiation resistance of *H. salinarum* (Robinson *et al.*, 2011; Webb *et al.*, 2013).

H. salinarum has multiple copies of the normal chromosome set, which is called polyploidy, depending on the growth phase (Breuert *et al.*, 2006). Although not all polyploidy cells show radiation resistance, the redundant copy of a gene in *H. salinarum* might complement the loss of genetic information induced by radiation exposure. A similar phenomenon is also observed in *D. radiodurans* (Hansen, 1978). According to transcriptome analysis in *H. salinarum*, expression of genes involved in the restoration of genome integrity increases during recovery process post-radiation exposure (Whitehead *et al.*, 2006). Interestingly, *H. salinarum* strains lacking Mre11 or Rad50 orthologs, which are essential components of the homologous recombination pathway, exhibit wild-type levels of resistance to γ-radiation and DNA damage insults (Kish and DiRuggiero, 2008). However, pulsed-field gel electrophoresis analysis revealed that *mre11*Δ and *mre11*Δ *rad50*Δ double mutants exhibit delayed DNA DSB repair without loss of viability compared to wild-type strain post-radiation exposure. This result implies that the DNA DSB repair machinery contributes to radiation resistance of *H. salinarum*.

Although *H. salinarum* has high intracellular concentration of KCl and multiple copies of the genome set, it remains elusive why this species shows remarkable radiation resistance. Therefore, enzyme-mediated ROS detoxifying systems are considered as one of the defense mechanisms against radiation exposure. However, *H. salinarum* strains lacking superoxide dismutase (SOD) or catalase exhibit wild-type levels of survival under ionizing radiation (Robinson *et al.*, 2011). Supporting this, expression levels of SODs and catalases are not significantly increased by radiation (Whitehead *et al.*, 2006). Instead, non-enzymatic antioxidant systems appear to play critical roles in the radiation resistance of *H. salinarum* (Kish *et al.*, 2009; Robinson *et al.*, 2011; Webb and DiRuggiero, 2012; Webb *et al.*, 2013). *H. salinarum* shows high Mn/Fe ratio (0.27) similar to that of *D. radiodurans* (Daly *et al.*, 2007; Kish *et al.*, 2009) and its extracts contain higher concentration of small molecules such as orthophosphate and low molecular weight peptides than those of radiation-sensitive strain, *E. coli* (Robinson *et al.*, 2011). The Mn^{2+}-inorganic molecules and -peptide complexes render cells to be resistant to radiation and oxidative stresses in bacteria and yeasts (Daly *et al.*, 2010; McNaughton *et al.*, 2010; Ghosh *et al.*, 2011). Supporting this notion, the protein-free ultrafiltrates from *H. salinarum* are able to protect protein activity, but not DNA structure, against ionizing radiation, which is similar to the case in *D. radiodurans* (Robinson *et al.*, 2011). Furthermore, the carotenoid pigment, bacterioruberin, which is responsible for bright pink and red color in *H. salinarum*, has hydroxyl radical scavenging activity, thereby protecting DNA structure from ionizing radiation (Saito *et al.*, 1997). Taken together, extreme ionizing radiation resistance of *H. salinarum* originates from a combination of the higher intracellular concentration of KCl and inorganic molecules, high ratio of Mn/Fe, and the presence of bacterioruberin.

Radiation-resistance mechanisms in fungi

Compared to bacteria and archaea, eukaryotes including animals and plants generally exhibit remarkable susceptibility to γ-radiation. However, of the eukaryotes, some fungi show

high radiation resistance (Saleh *et al.*, 1988). The filamentous fungus *Alternaria alternata* is found in highly radioactive environments such as a reactor of the Chernobyl nuclear power plant (Mironenko *et al.*, 2000). One of the radiation-esistance factors in *A. alternata* is a black pigment melanin, which is composed of polymerized indole and phenolic compounds and accumulated in mycelium (Kimura and Tsuge, 1993). Supporting this notion, melanized fungi dominate other fungi at radiation-polluted areas near the Chernobyl nuclear power plant and inhabit the walls of the radioactive contaminated reactor (Vember and Zhdanova, 2001; Zhdanova *et al.*, 2004).

Cryptococcus neoformans is another radiation-resistant fungus. It is a basidiomycete fungal pathogen that causes life-threatening meningoencephalitis in immunocompromised populations (Idnurm *et al.*, 2005). *C. neoformans* produces the black pigment, melanin, which is catalyzed by laccases and is considered as an important virulence factor (Kwon-Chung *et al.*, 1982; Williamson, 1994). In addition to pathological property, *C. neoformans* exhibits high resistance to radiation similar to other melanized fungi (Dadachova and Casadevall, 2008). Recent studies revealed that diverse biological and chemical properties of melanin contribute to the radiation-resistance of *C. neoformans* (Bryan *et al.*, 2011; Khajo *et al.*, 2011; Pacelli *et al.*, 2017). Melanin appears to mediate energy transduction during radiation exposure. Dadachova *et al.* (2007) demonstrated that melanized fungal cells of *C. neoformans* and *Wangiella dermatitidis* show increased growth rate relative to their non-melanized cells under radiation exposure. They found that ionizing radiation changes the electron transfer properties of melanin through the measurement of NADH-ferricyanide redox reaction. This result indicates that *C. neoformans* melanin could employ γ-radiation as an energy source by converting electromagnetic energy into chemical energy. This phenome is linked to "radiotropism." Zhdanova *et al.* (1991) previously reported that fungal samples extracted from a radioactive environment exhibit growth toward the direction of radiation source. They assumed that melanin in fungi plays roles similar to those of chlorophyll in phototrophic plants by absorbing γ-ray as electromagnetic energy and then changing it to chemical energy that fungi can utilize. Besides its energy transducing role, melanin protects cells from diverse environmental stresses such as heavy metals, oxidative damage, and UV irradiation (Nosanchuk and Casadevall, 2003). Melanin contributes to radiation resistance by scattering photons and electrons as a radioprotective barrier and quenching radiation-induced ROS (Dadachova *et al.*, 2008). Taken together, fungal melanin is an essential factor for radiation resistance.

Notably, *C. neoformans* can synthesize melanin when supplied with exogenous substrates, whereas *W. dermatitidis* produces melanin intrinsically. This fact indicates that other inherent cellular factors might contribute to radiation resistance of *C. neoformans*. Recently, Jung *et al.* (2016) performed transcriptome analysis to elucidate the radiation-resistance mechanism of *C. neoformans* and found that a substantial proportion of *C. neoformans* genes (37% of a total of 6,962 genes) exhibits differential expression patterns post-γ-radi-

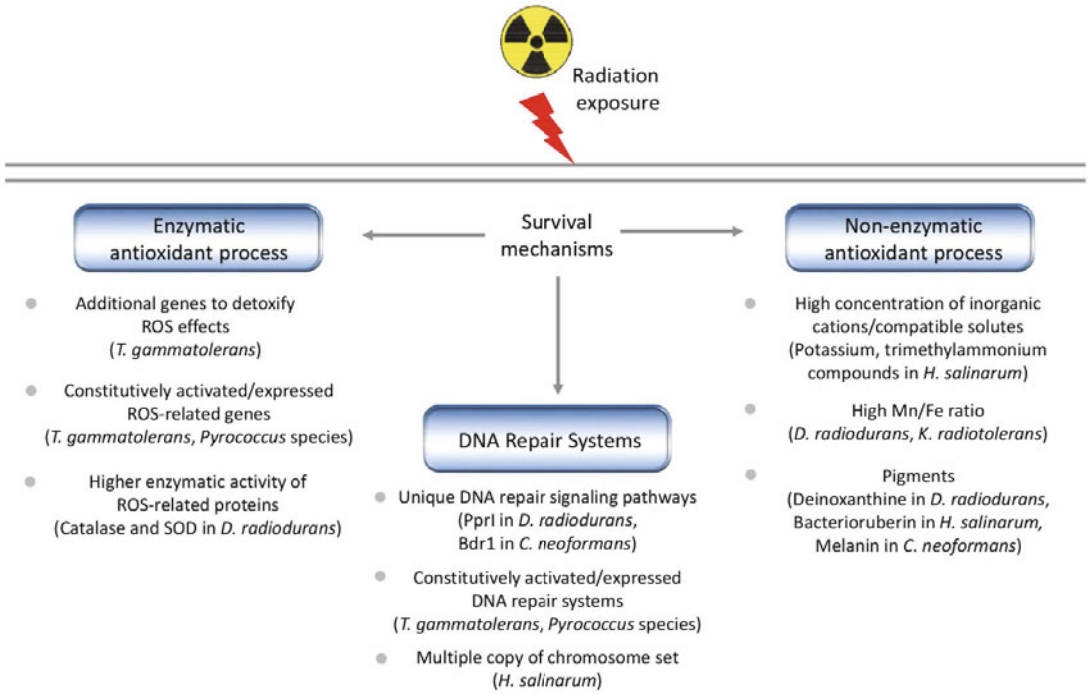

Fig. 1. Potential mechanisms for microbial radiation resistance. Upon radiation exposure, microorganisms employ diverse defense systems to alleviate deleterious effects. In the aspect of DNA repair systems, some microbes possess unique DNA repair signaling components, constitutively activate or express DNA repair machineries, or harbor the polyploidy chromosome set. To protect cells from oxidative stress generated by radio-hydrolysis of water, microorganisms not only utilize enzymatic antioxidant systems with higher activity or expression of ROS-related proteins, but also non-enzymatic antioxidant systems with high intracellular concentration of inorganic solutes, high Mn/Fe ratio, and pigments.

ation exposure. According to the functional categories, genes involved in DNA replication and repair, post-translational modification, protein turnover, and chaperone function are highly up-regulated in the early recovery time, whereas genes involved in translation, amino acid metabolism, and transport are down-regulated in the late recovery time. This transcriptome profile is similar to that of radiation-sensitive fungi, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Gasch *et al.*, 2001; Watson *et al.*, 2004). Furthermore, Jung *et al.* (2016) found that *C. neoformans* lacking the evolutionarily conserved genes involved in DNA repair process, the oxidative stress response and protein folding pathway show radiation sensitivity, similar to radiation-sensitive fungi. Notably, however, they found that expression of the unique bZIP transcription factor Bdr1, which is found only in the *Cryptococcus* species, is significantly increased post-radiation exposure. Bdr1 was shown to play critical roles in γ-radiation resistance by controlling expression of DNA repair genes (Jung *et al.*, 2016). Nevertheless, the γ-radiation-resistance mechanism mediated by Bdr1 remains elusive. Previous study revealed that expression levels of *BDR1* are controlled by the evolutionarily conserved DNA damage response kinase, Rad53 (Jung *et al.*, 2016). Moreover, Rfx1 transcription factor, which is evolutionarily conserved downstream of Rad53 in eukaryotes, is not found in *Cryptococcus* species. Therefore, Bdr1 might be a functional homologue of Rfx1 transcription factor. To further address Bdr1-mediated radiation-resistance mechanism, the Bdr1-specific regulons and its upstream regulators and downstream targets should be investigated at the genome-scale.

Given that radiation-resistance mechanisms in fungi have not been extensively studied compared to radiation-resistant species in bacteria and archaea, examination of protein carbonylation levels, DNA repair efficiency, and Mn/Fe ratio between radiation-resistant and sensitive fungi may provide additional insights about fungal radiation-resistance mechanisms.

Conclusion

In this present review, we described and discussed how microorganisms of three domains of life overcome the harmful effects caused by radiation exposure as summarized in Fig. 1. Notably, 'protective mechanisms' and 'efficient repair systems' are generally accepted as strategies for surviving under high dose of radiation exposure. 'Protective mechanism' refers to the manner in which cells protect themselves from radiation exposure by constitutively activating or expressing antioxidant and DNA repair systems, and by producing intracellular pigments and solutes. 'Efficient repair systems' refers to the more efficient repair machineries of DNA and antioxidant systems in these cells compared to those in radiation-sensitive organisms, thereby surviving without loss of viability post-radiation exposure. Recently, there are efforts under way to apply biological products, including proteins and metabolites, generated by radiation-resistant organisms to commercial uses such as anticancer drug, antioxidant, and sunscreens in the biotechnology industry. For example, Mn^{2+}-peptide complex from *D. radiodurans* enhances vaccine efficiency by preserving bacterial and viral epitope during radiation vaccine development (Gaidamakova *et al.*, 2012). Furthermore, our group generated stress-tolerant *E. coli* for industrial biotechnology by introducing *Deinococcus* genes (Appukuttan *et al.*, 2015; Park *et al.*, 2016). Therefore, comprehensive understanding of microbial radiation-resistant mechanisms will not only shed light on how living organism survive in the extreme environmental condition, but also pave the way for potential usage of radiation-resistant microbes or related biomolecules for human benefits.

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